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VLP sequencing

Kate L. Jeffrey

Updated date: May 4, 2022

An abbreviated version of this protocol was published in Science Immunology in Apr 2022

Human enteric viruses autonomously shape inflammatory bowel disease phenotype through divergent innate immunomodulation

DOI: 10.1126/sciimmunol.abn6660

Detailed protocol

Virus-like Particle (VLP) isolation

100 mg of colon tissue or centrifuged ileostomy fluid was resuspended in 500 mL of saline magnesium buffer (SM) buffer (8 mM $MgSO_4$, 100 mM NaCl, 50 mM Tris-HCl pH 7.4, and 0.002% (w/v) gelatin, passed through a 0.02mm Whatman filter), homogenized using sterile metal beads, and centrifuged. The supernatant was then passed through a 0.45 µm pore-sized membrane, followed by 0.22 µm pore-sized membrane (Millipore). The filtrates were treated with lysozyme (10 mg/mL; Sigma) for 30 min at 37 °C, followed by incubation with 0.2 volumes of chloroform for 10 min to degrade any remaining bacterial and host cell membranes and then centrifuged at 2,500 × g for 5 min at room temperature. The aqueous phase was collected and incubated with DNase I (3U/200mL, Sigma) for 1 h at 37 °C to remove any non-virus protected DNA. Enzyme activity was inactivated by incubation at 65°C for 15 min as described (*1*). To remove any potential residual endotoxin, samples were treated with Polymyxin B (10 mg/mL, Sigma) for 30 minutes. Undetectable endotoxin was confirmed using Limulus Amebocyte Lysate (LAL) assay kit (GenScript) following the manufacturer's instructions.

Quantification of VLPs

Virus-like particles (VLPs) were diluted 10-fold serially, stained for 30 minutes with 10× SYBR Gold (Thermo Fisher Scientific) for nucleic acid or the dialkylcarbocyanine Dil (Thermo Fisher Scientific) for lipid bilayers of enveloped viruses, and imaged using a Zeiss LSM510 laser scanning confocal microscope. Images were captured using Zeiss software (ZEN). Particles <0.5 µm in diameter were regarded as VLPs. SM buffer was used as a negative control. 10 VLP images were captured per sample and VLPs were counted using an image analyzer (InnerView™).

VLP sequencing

Total RNA and DNA was extracted from VLPs on a MagNA Pure 24 instrument (Roche), according to the manufacturer's instructions. In order to evaluate samples for both RNA and DNA viruses, the total nucleic acids were randomly amplified as described previously (*65, 66*) using barcoded primers consisting of a base-balanced 16-nucleotide-specific sequence upstream of a random 15-mer and used for NEBNext library construction (New England Biolabs). The libraries were multiplexed on an Illumina NextSeq (Washington University Center for Genome Sciences) using the paired-end 2 × 150 protocol.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Jeffrey, K. (2022). VLP sequencing. Bio-protocol Preprint. bio-protocol.org/prep1652.
2. Adiliaghdam, F., Amatullah, H., Digumarthi, S., Saunders, T. L., Rahman, R., Wong, L. P., Sadreyev, R., Droit, L., Paquette, J., Goyette, P., Rioux, J. D., Hodin, R., Mihindukulasuriya, K. A., Handley, S. A. and Jeffrey, K. L.(2022). Human enteric viruses autonomously shape inflammatory bowel disease phenotype through divergent innate immunomodulation. Science Immunology 7(70). DOI: 10.1126/sciimmunol.abn6660